Interestingly, despite full triad targeting, β_3 was unable to restore considerable charge movement (Q_{max}, 2.53 ± 0.50 nC/μF) in contrast to the other β isoforms (Q_{max}, 8.86 ± 0.93 to 9.94 ± 2.06 nC/μF) upon expression in *relaxed* myotubes. Systematic exchanges of variable regions and conserved domains of β_{1a} with corresponding β_3 sequences revealed significantly reduced Q_{max} restoration with SH3 and C-terminal chimeras (Q_{max}, 4.02 ± 0.28 and 5.57 ± 0.74 nC/μF, respectively). In contrast, β_{1a}/β_3 chimeras with the N-terminus, HOOK and GK domain exchanged showed complete restoration of charge movement.

Together, our data suggest an essential role of the conserved SH3 domain and the variable C-terminus of β_{1a} in the induction of the voltage-sensing function of the DHPRα_{1S} in skeletal muscle EC coupling.

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3200-Pos Board B305

Troponin T3 Regulates Calcium Channel Beta1a Subunit Nuclear Translocation in Skeletal Muscle

Tan Zhang, Jackson Taylor, Zhongmin Wang, Osvaldo Delbono.

Voltage-dependent calcium channel (VDCC) beta subunits (CaVβ) are critical for CaVα subunit membrane expression and gating properties. While CaVβ interacts with a variety of other molecular partners, nonchannel functions of skeletal muscle CaVβ1a have not been reported. In a yeast two hybrid (Y2H) screening of the mouse tibialis anterior (TA) muscle cDNA library, troponin T3 (TNNT3), specific to fast skeletal muscle, was identified using full-length CaVβ1a as the bait. Further studies revealed that TNNT3, but not the slow skeletal muscle TNNT1, co-immunoprecipitated with CaVβ1a in the mouse skeletal muscle cell line C2C12 and mouse skeletal muscle. Consistently, both CaVβ1a and TNNT3 were highly expressed in mouse fast, but not slow, skeletal muscle. Surprisingly, when DsRed-tagged TNNT3 and YFP tagged CaVβ1a (CaVβ1a -YFP) were transiently co-expressed in C2C12 cells or the mouse flexor digitorum brevis (FDB) muscle, both showed a punctuate distribution pattern in the cytoplasm and nucleus. In comparison, CaVβ1a-YFP alone localized uniformly in the cytoplasm. Further truncation analysis revealed that TNNT3 C-terminus (aa 161-244) localized exclusively in FDB nuclei and enriched CaVβ1a -YFP accordingly. In contrast, the TNNT3 middle region (aa 55-160) localized only in the cytoplasm, while the N-terminus (aa 1-54) was found in both cytoplasm and nucleus. Note that the N-terminus could also recruit CaVβ1a-YFP in the nucleus. Y2H assay verified that the TNNT3 C-terminus has the strongest interaction with CaVβ1a. Immunoblotting with an antibody targeting the TNNT3 N-terminus detected an increased TNNT3 fragment in the aging mouse skeletal muscle. We conclude that TNNT3 interacts with CaVβ1a and regulates its nuclear translocation mainly through its TNNT3 C- or N-terminus. These findings strongly support a transcription-regulation function for CaVβ1a and TNNT3.

3201-Pos Board B306

ATP Sensitivity and IP_3-Dependent Calcium Transients Which Regulate Gene Expression in Adult Muscle Fibers are Altered in Mdx Mice

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ATP has been shown to be released from muscles during exercise and after tetanic electrical stimulation (ES) and induces IP_3 dependent slow calcium transients involved in gene expression in myotubes. The aim of this study was to characterize these signals in adult fibers and to establish differences between normal and mdx mouse.

Experiments were performed in cultured isolated muscle fibers obtained from normal and mdx adult mice. The release of ATP induced by ES was measured using luciferin-luciferase and for calcium release studies the fibers were loaded with fluo-3. The location and expression of the nucleotide receptors were determined by immunofluorescence and western blot.

Slow, post tetanic calcium signals were partly inhibited by pharmacological agents targeting pannexin-1 (a pathway for ATP release), purinergic receptors and extracellular ATP. These signals had slower decay constants in mdx fibers. ES induced significant ATP release in normal fibers but this release was not detected in mdx fibers. The cellular location of ATP receptors was similar in both fiber types but their level of expression was higher in mdx fibers. Pannexin-1 expression was significantly augmented in mdx fibers. External ATP induced Ca^{2+} signals in both, normal and mdx muscle fibers, but the sensitivity to ATP was much higher in mdx fibers. This study demonstrates that ATP signaling is profoundly altered in mdx fibers and could be implicated in the calcium disturbance described in DMD; a compensatory mechanism may be involved in the reduced ATP extrusion apparent in dystrophic cells.

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3202-Pos Board B307

The Role of Ryanodine Receptor Phosphorylation in Skeletal Muscle Excitation-Contraction Coupling

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Activation of the type 1 ryanodine receptor (RyR1) during skeletal muscle excitation-contraction (EC) coupling allows the release of stored Ca^{2+} required for muscle contraction. While RyR1 is a well-known substrate of protein kinase A (PKA), the physiological significance of this phosphorylation event is poorly defined. PKA is known to increase the open probability of the channel in lipid bilayers by inducing phosphorylation of a specific residue (S2844). Knock-in mice harboring phosphorylation site deficient (RyR1-S2844A) and phosphomimetic (RyR1-S2844D) mutations were generated in order to probe the consequence of this phosphorylation event in skeletal muscle EC coupling. Specific force measurements were performed on extensor digitorum longus (EDL) muscles and intracellular Ca^{2+} release was examined in isolated flexor digitorum brevis (FDB) muscle fibers using confocal microscopy. While isoproterenol (ISO) enhanced both Ca^{2+} release and muscle force in wild type (WT) mice, the positive effects of ISO were abrogated in samples from RyR1-S2844A mice. This demonstrates the enhancing effects of RyR1 phosphorylation during adrenergic stimulation in skeletal muscle. While transient phosphorylation of RyR1 exerts positive effects, chronic phosphorylation, as occurs in animal heart failure models, is thought to be detrimental to muscle function. We tested this hypothesis by measuring muscle force production and intracellular Ca^{2+} in RyR1-S2844D mice. At 3-months of age, muscle function and Ca^{2+} release were similar to WT controls. However, muscle force production and intracellular Ca^{2+} release were both blunted by 6-months of age suggesting that a pathological Ca^{2+} leak induced by the S2844D mutation impairs muscle function.

3203-Pos Board B308

Nox2 Dependent Modulation of Skeletal Muscle EC Coupling

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Generation of reactive oxygen species (ROS) under physiological conditions is required for normal force production in skeletal muscle. However, high levels of ROS promote contractile dysfunction, resulting in muscle weakness and fatigue. Our recent studies suggest that sub-cellular site-specific ROS production governs the beneficial vs. damaging effects of ROS. NADPH oxidase (Nox2) is an enzyme complex that generates ROS. In this study we investigated the role of Nox2 in skeletal muscle EC coupling. Using sub-cellular site-specific redox probes we show that cytosolic glutathione redox potential becomes more oxidized during contractile activity while mitochondrial redox potential does not change. We show that increased contractile activity promotes Nox2 dependent ROS production. Interestingly, we have found that the non-tyrosine kinase Src is activated in response to increased contractile activity. These data support a hypothesis in which Src acts as a redox switch to activate Nox2. Increased ROS production during strenuous exercise would decrease sarcolemmal Ca^{2+} influx and decrease sarcoplasmic reticulum refilling, which could contribute to the development of fatigue. As trials of general antioxidant therapy to combat increased ROS production have not been convincingly beneficial, understanding the sub-cellular signaling pathways by which oxidants influence muscle function will allow for the development of targeted therapeutic interventions to combat the deleterious effects of sustained contractile activity as well as skeletal muscle diseases.

3204-Pos Board B309

Pathological RyR1 Mutations to Identify RyR1 Functional Domains

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Muscle contraction is achieved when an efficient excitation signal at the plasma membrane triggers intracellular calcium release. This process called ‘‘excitation-contraction (E-C) coupling’’ relies on a multimolecular protein complex, the calcium release complex. This complex, spanning the plasma membrane and the sarcoplasmic reticulum (SR), is organized around the calcium channel of the SR, the ryanodine receptor (RyR1).

Mutations in RyR1 lead to a number of muscle diseases: Central core disease (CCD), Multi mini core disease (MmD), centronuclear myopathy, Malignant hyperthermia¼ Nevertheless, the functional consequences of each mutation are largely unknown because of the lack of information on the functional domains in RyR1. For the present study, we use the mutations identified in RyR1 to decipher functional regions in RyR1. We have identified more than 200 RyR1 mutations in the course of clinical diagnosis. We have recently shown that RyR1 interacts with caveolin-3, via the transmembrane part of the channel (Vassilopoulos et al, 2010, Biochemistry, 49, 6130). Caveolin-3, which is a structural protein involved in intracellular trafficking, could therefore regulate RyR1 targeting. We are now searching for mutations in RyR1 which could affect the